

Scotland's Rural College

Assessment of circadian rhythm of activity combined with random regression model as a novel approach to monitoring sheep in an extensive system

Sarout, BNM; Waterhouse, A; Duthie, C-A; Poli, CHEC; Haskell, MJ; Berger, A; Umstatter, C

Published in:

Applied Animal Behaviour Science

DOI:

[10.1016/j.applanim.2018.06.007](https://doi.org/10.1016/j.applanim.2018.06.007)

First published: 28/06/2018

Document Version

Peer reviewed version

[Link to publication](#)

Citation for pulished version (APA):

Sarout, BNM., Waterhouse, A., Duthie, C-A., Poli, CHEC., Haskell, MJ., Berger, A., & Umstatter, C. (2018). Assessment of circadian rhythm of activity combined with random regression model as a novel approach to monitoring sheep in an extensive system. *Applied Animal Behaviour Science*, 207, 26 - 38.
<https://doi.org/10.1016/j.applanim.2018.06.007>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Assessment of circadian rhythm of activity combined with random regression model as a novel approach to monitoring sheep in an extensive system

Bruna Nunes Marsiglio Sarout ^{a, b, *} Anthony Waterhouse ^a, Carol-Anne Duthie ^a, Cesar Henrique Espirito Candal Poli ^b, Marie J. Haskell ^a, Anne Berger ^c, Christina Umstatter ^d

^a Scotland's Rural College, West Mains Road, Edinburgh, EH9 3JG, Scotland, UK

^b Universidade Federal do Rio Grande do Sul, Faculdade de Agronomia, Av. Bento Gonçalves 7712, Porto Alegre, 91540-000, Rio Grande do Sul, Brazil

^c Leibniz-Institute for Zoo and Wildlife Research, Alfred-Kowalke-Str. 17, 10315 Berlin, Germany

^d Agroscope, Tänikon 1, 8356 Ettenhausen, Switzerland

* Corresponding author: Bruna.Sarout@gmail.com

E-mail addresses: Bruna.Sarout@gmail.com (B. N. Marsiglio-Sarout), Tony.Waterhouse@sruc.ac.uk (A. Waterhouse), Berger@izw-berlin.de (A. Berger), Carol-Anne.Duthie@sruc.ac.uk (C-A. Duthie), cesar.poli@ufrgs.br (C. H. E. C. Poli), Marie.Haskell@sruc.ac.uk (M. Haskell), christina.umstaetter@agroscope.admin.ch (C. Umstaetter).

Abstract

Sensor-based technologies are becoming increasingly available and can be used to automatically gather long-term data about animal behaviour. With this information, it is possible to assess the circadian rhythm of activity and monitor its response to internal and external factors. Identifying irregularities in this rhythm may indicate animal health and welfare issues. The aim of this study was to collect sensor-based general activity and investigate circadian rhythm of this activity to identify the changes due to weather influences that act on these parameters throughout the year; to identify the differences between individuals; and to assess links between general activity and circadian rhythm of activity with sheep body weight change. In total, 29 Scottish Blackface Degree of Functional Coupling (DFC)

ewes of different ages and body condition scores were used. The animals were monitored for four consecutive weeks in each of four seasonal periods, in extensive systems on Scottish upland pastures, and without human handling during study periods. Accelerometer-integrated collars were fitted to the animals to collect the motion index continuously every minute. These data were used to calculate the percentage of cyclic behaviour that was harmonic/ synchronized with the environment (over 24 h period), as Degree of Functional Coupling (DFC). The DFC was shown within rolling seven-day periods. Low DFCs indicate low synchronization. Weather data were collected daily. Random regression models were used to assess between-individual variation. During the winter period, the level of the DFC for the activity of nineteen ewes lowered in response to a period of high level of precipitation combined with the low winter temperatures. However, four ewes exhibited a lower level of variation in the DFC values, showing that there were differences between individuals in regard to their response to the precipitation level. The overall mean of the DFC for the general activity was highest in autumn (95.4%, $P < 0.001$), however, it did not differ between summer and spring (respectively 90.2% and 88.1%, $P > 0.05$), but was significantly lower during the winter (81.7%, $P < 0.001$) compared with summer and autumn. Over the spring and summer, variation in DFC was a good estimator of body weight gain. It was concluded that the assessment of circadian rhythms of general activity using the DFC-parameter allows a better understanding of sheep responses to weather influences, compared to the evaluation of general activity alone. The random regression model method was effective in identifying animals that deviated positively or negatively from population responses.

Keywords: between-individual variation; Degrees of Functional Coupling; phenotypic plasticity; precision livestock; sheep performance; seasonal adaptation.

1. Introduction

In extensive production systems, animals are often kept in semi-natural habitats without close supervision for long periods. The use of a telemetric monitoring system could be very important to ensure health and welfare, and provide management information for the animals.

Use of ICT (Information and Communication Technology) to optimise management processes in livestock production has increased in recent years. Major developments can be found in intensive production systems, particularly with the direct use of remotely collected data on behavioural traits, such as the use of activity data to detect oestrus in dairy cows (Firk et al., 2002). Technologies such as accelerometer-based sensors enable us to measure animal behaviour, for example, general activity, and these continuous data can be used to calculate parameters describing the rhythmic structure of behaviour.

Biological processes display an endogenous oscillation of about 24 hours, known as circadian rhythms (Foster and Kreitzman, 2005; Koukkari and Sothorn, 2006; Piccione et al., 2005; Refinetti and Menaker, 1992; Wood and Loudon, 2014). Many basic behaviours, such as sleep and activity, are under the control of these rhythms. The rhythms are controlled by genes activating proteins and neurotransmitters (Foster and Kreitzman, 2005; Wood and Loudon, 2014). Although these biological processes are endogenous, they are adjusted to the local environment by circadian cues (e.g. light, temperature and humidity) (Foster and Kreitzman, 2005; Lincoln and Richardson, 1998). Thus, the circadian rhythm of behavioural variables is an outcome of both endogenous and exogenous motivational factors (Foster and Kreitzman, 2005; Scheibe et al., 1999). Accordingly, Scheibe et al. (1999) developed a parameter called Degree of Functional Coupling (DFC) to characterise the circadian rhythm of behavioural variables. Therefore, DFC expresses the percentage of the measured behaviour that is harmonically synchronized with environmental rhythms, over a 24-hour period.

Valuable information may be obtained by looking at the synchrony between behavioural and environmental rhythms. A considerable number of studies has shown a link between environmental influences or health issues with the level of DFC for activity of free-ranging animals (Berger et al., 2003, 1999, Scheibe et al., 1999, 1998), with a strong circadian rhythm of activity known to be a characteristic of healthy and adapted organisms (Berger et al., 2003, 1999; Bloch et al., 2013). Thus, the rhythmicity of activity is a variable that has clear potential to be a key indicator of the state of animals. This suggests that the evaluation of the DFC would provide useful information as part of an assessment of animal welfare in farmed livestock. Until

recently, continuous 24/7 monitoring of animal activity was not cost-effective. However, the rapid development of new technologies makes it possible to look a new way of using this type of data.

The rhythmicity of activity of each animal is unique and may vary according to short- and long-term environmental influences or its physiological status (Berger et al., 2003; Scheibe et al., 1999). Therefore, an in-depth look at the within- and between-individual behavioural rhythmicity is important to understand at an individual level whether there are relationships between the rhythmicity of activity, environmental influences and production traits. However, to fully understand the complex behavioural variation between individuals within a single population is not a simple task, a large number of recent studies have focussed on how to measure these between-individual differences (Dingemanse et al., 2010; Dingemanse and Wolf, 2013; Herczeg and Garamszegi, 2012; Nussey et al., 2007; Sih et al., 2004).

Many evolutionary ecology studies have used simple linear regression models to understand and identify the population level response to the environment, at both phenotypic and genetic levels. In this model, the coefficient of the linear regression of the phenotype (for example behaviour) with the environmental variable is described as the ‘reaction norm’ (Pigliucci, 2001). However, a study by Nussey et al. (2007) asserted that little is known about the prevalence, and evolutionary and ecological causes and consequences of the variation in life history phenotypic plasticity (the ability of individuals to adapt their phenotypic traits in response to the environment) in the wild. Considering this, Nussey et al. (2007) outlined an analytical framework using the reaction norm concept and random regression models to access not only the population level responses but also the between-individual variation of labile traits. Labile traits are those that easily alter over an individual lifetime, e.g. those related to physiology or behaviour (Nussey et al., 2007). In order to understand the between-individual variation of activity rhythmicity, this study combines for the first time the DFC analysis for each animal with random regression models.

Sheep have seasonal production cycles and often occur in bio-geographic zones with large shifts in grassland quality and availability and light/dark cycles (Lincoln et al., 1990;

Lincoln and Richardson, 1998). Extensive systems throughout the world have high levels of variation in environmental and nutritional impacts upon the farmed livestock. Scottish extensive systems pose particularly demands upon sheep, where winter periods of poor grazing nutrition, short day lengths, variable and extreme weather and pregnancy overlap. Poor welfare and high rates of mortality are risks within these systems, and the sheep are often inspected infrequently with limited potential for intervention (Morgan-Davies et al., 2008).

The aim of this study was to analyse activity data (through a motion index) and the circadian rhythm of this activity to identify changes due to weather influences that act on these parameters throughout the year; to identify the differences between individuals; and to assess links between general activity and circadian rhythm of activity with sheep body weight gain. The study was carried out with an experimental cohort of animals, kept in a challenging environment, representative of the challenges faced by the wider population of farmed sheep in extensive systems. Our study has the novel approach of addressing the key issue of whether the calculation of the circadian rhythm of general activity using the DFC-parameter better explains sheep behaviour and performance compared to general activity measured through the motion index.

2. Material and Methods

This experiment was conducted at Scotland's Rural College (SRUC) Hill & Mountain Research Centre. The experimental protocol was approved by SRUC's Animal Welfare and Ethical Review Body, the Animal Experiments Committee, and was conducted in accordance with the requirements of the United Kingdom Animals (Scientific Procedures) Act, 1986.

2.1. Animal measurements

The study was done on a group of 29 Scottish Blackface ewes (Supplementary Table 1). The ewes covered a range of ages (two to six years old) with a body condition score (BCS) ranging from 2 to 3 points at the start of the study. The BCS scale ranges from 1 to 5 where 1 is thin and 5 is fat. The ewes were kept on 23.6 ha of semi-natural pasture in the West Highlands

of Scotland (SRUC, Hill & Mountain Research Centre, Scotland), with a latitude and longitude of 56.4381 and -4.6684. The annual precipitation is typically 2800 mm. A UK Meteorological Office Weather Station, based on the research farm and a distance of < 2 km away from the field location, measured the precipitation and temperatures every day. Over all seasons the ewes were kept under natural conditions and no formal built shelter was provided. Human contact was avoided, except for the daily visual checks over all seasons and feed provision over the winter. Ewes were closely checked at the start and end of each measurement period. Endpoint criteria for a potential exclusion of an animal from the experiment were in place according to the ethical review body of SRUC, and no sheep fell below body condition score limits. During the winter period, ewes were supplemented with two large round hay bales, one provided on the 17th of January and the other on the 5th of February. Energy and protein feed blocks (Rumevite Sheep, Rumenco, Burton-on-Trent, UK) were also provided, at a rate of one per week, each weighing 20 kg. The supplementation and the adjacent grazing was representative of that given to the larger flock from which the experimental cohort were drawn. Overall levels of grass quality and quantity and supplementation provided were judged to be somewhat higher than the larger flock, but the experimental sheep, being within a smaller area had less choice in terms of grazing area and moving to natural shelter features. In spring, summer and autumn, the ewes were exclusively grazed on semi-natural pasture.

Sheep activity data were continuously collected by three-axes accelerometers (IceTag Pro, IceRobotics Ltd., Edinburgh, Scotland) integrated into a collar and fitted to the ewes. Activity data were recorded over four consecutive weeks in each season, across a full annual cycle, commencing in the winter. The collars were changed every period and, thus, ewes were wearing different Icetag Pro loggers during different seasons to ensure any systematic differences between collars were not confounded with the animal. The IceTag Pro logger is programmed to record the g-force in three dimensions (IceRobotics Ltd, Product Guide 2010), providing the motion index. Motion index is the average of the magnitude of acceleration on each of the three axes for each minute. A low motion index corresponds to a low activity level

and a high motion index corresponds to a high activity level. Weight and condition scores of ewes were taken at the beginning and end of each seasonal measuring period.

Twenty-four ewes were measured in each of the four seasons. The ewes used for this study were drawn from a larger flock and kept as a sub-group but in a restricted area. An additional, seven ewes without collars were also included in the monitoring group to ensure replacement when necessary. As some data were missing, the number of collected data sets differed between seasons. In the winter period twenty-four ewes commenced the study with loggers, however, there was missing logger data for one ewe due to technical failure. There were two mortalities during the winter measurement period, and one mortality a few days after the end of measurements (non-experimental period). Three replacement ewes were used in the spring experimental period. Over the summer period, there were missing logger data from five ewes due to technical failures. Over the entire experiment, data from twenty-nine ewes were used, and fifteen ewes had data from all four seasons.

Winter measurements resulted in twenty-three data sets from ewes during mid-pregnancy (12th of January to 10th of February), under natural winter conditions, with sunrise between 07:57 – 08:46 h and sunset between 16:08 – 17:09 h. Spring measurements were performed on 22th of May to 18th of June, after parturition around the 1st of May. Out of the twenty-four ewes, sixteen were rearing lambs (six with twin and ten with single lambs) and eight ewes were without lambs. Ewes and lambs were under natural spring conditions, with sunrise between 04:28 – 04:51 h and sunset between 21:39 – 22:11 h. Summer measurements were collected during late lactation (1st to 28th of August). Data were successfully collected from nineteen ewes, of which twelve were rearing lambs (six ewes with twin and six with single lambs). Ewes and lambs were kept under natural summer conditions, with sunrise between 05:20 – 06:14 h and sunset between 20:26 – 21:30 h. Lamb weaning took place at the end of the summer period. Autumn measurements were collected one week after weaning and before mating (5th to 30th of October). Twenty-four data sets were collected from ewes in autumn, with sunrise between 07:30 – 08:22 h and sunset between 17:42 – 18:45 h. At the beginning and at the end of each experimental period the ewes were weighed in order to evaluate Body Weight Gain (BWG).

2.2. Data analysis

2.2.1. Activity circadian and ultradian rhythms

DFC is a parameter, developed by Scheibe et al. (1999), used here to measure the synchrony between circadian rhythm of behaviour and circadian rhythm of the environment (external 24 h period). Thus, DFC expresses the percentage of cyclic behaviour that is harmonically synchronized to the 24 h period. Any analysis of cyclical data involves fitting mathematical functions and these are described in sequence.

To carry out the DFC analyses, preliminary analysis using different time intervals (e.g. 1, 5, 10, 15, 20 and 30-minute intervals) and different day intervals (e.g. 7 and 10 days) were performed, to identify the appropriate interval that best represented the patterns within the motion index data. Fifteen-minute time intervals and seven-day periods of data were the most appropriate and have been used throughout this paper. Therefore, time series with fifteen-minute intervals were created, by taking the motion index sum of the one minute averages, and then using the method of Scheibe et al. (1999), these data were analysed for its rhythmic components.

To reduce the noise component, autocorrelation functions were calculated for each seven-day interval, with a shift by one day and overlap of six days. Thus, the first seven-day interval included the first day to the seventh day of measurement; the second seven-day interval included the second day to the eighth day of measurement and so on. A power spectrum was drawn for each seven-day interval from the autocorrelation functions of the measured data. The periodogram ordinates were calculated for all Fourier frequencies: $\omega=2\pi j/n$, $j=1, \dots, q$ with $q=n/2$ (n even) or $q=n/2-1$ (n odd), with n being the number of data points in the sample (Berger et al., 1999; Scheibe et al., 1999). The periodogram ordinates were tested for statistical significance according to the R.A. Fisher test for periodicity (Andel, 1984). The significant ordinates represent the significant periodic components (Supplementary Figure 1, a).

Harmonic periods are defined as periods which are synchronized with the day length (24 h) in relation to an integral number (1, 2, 3... etc.). Thus, 24 h divided by an integer number

gives the harmonic periods (the period lengths of 24, 12, 8, 6, 4.8, 4, 3.4, 3, 2.6, etc. hours are harmonic). An example of a power spectrum and its significant harmonic periods is shown in Supplementary Figure 1, b. Here, DFC is expressed as the percentage of the circadian components and harmonic ultradian components in relation to all rhythmic components of the spectrum. Therefore, DFC demonstrates the relationship between the total intensity of significant periods that were harmonic to the circadian period (SI_{Harm}) and the total intensity of all periods that were significant (SI_{Total}) (Berger et al., 1999; Scheibe et al., 1999):

$$DFC (\%) = (SI_{Harm} * 100) / SI_{Total}$$

Where, $SI_{Harm} = \Sigma$ of intensities of significant periods that are harmonic to 24 hours period;

$SI_{Total} = \Sigma$ of intensities of significant periods.

DFC (%) varies from 0% to 100%, where 0% means that only non-harmonic periods were significant and 100% means that only harmonic periods were significant (Berger et al., 2003, 1999). Low DFC indicates lower synchronization and high DFC higher synchronization to the 24-hour period. DFC was continuously calculated for all ewes, during all experimental days.

2.2.2. Statistical analysis of differences between seasons

The averages of BWG, motion index, DFC, standard deviation (STD) of DFC and STD of motion index for each season were calculated. Data were checked for normal distribution using the PROC UNIVARIATE statement of SAS 9.3. The DFC and STD of DFC averages for seasons were not normally distributed; hence, angular transformations were used. The motion index and STD of motion index averages for seasons were also not normally distributed; thus, square root transformations were used. To assist with interpretation of biological meaning, back-transformed means are presented in the results section. The effect of season was analysed using the MIXED Procedure of SAS 9.3, with season as repeated measure and ewe as random effect. The *P* values of the differences of least square means were adjusted for Tukey-Kramer and the significance level considered was 5%.

The general activity patterns of ewes during a 24-hour period in each season were calculated, by taking the overall average of motion index for all ewes for every minute. The threshold to consider the motion index per minute as an activity bout was the value of 1. As there were large differences between seasons in the 24-hour general activity patterns and DFCs averages, the subsequent analyses were done for each of the seasons separately.

2.2.3. Within seasons statistical analyses

2.2.3.1. Overall links between weather variables, general activity and circadian rhythm of activity

Regressions between overall daily averages (of all ewes) of motion index and its STD in relation to daily weather variables were calculated for each season; however, only significant and relevant results are shown in the results section. Each DFC for activity was calculated within moving seven-day intervals, thus, to allow juxtaposition, the moving averages of weather variables were also set up in seven-day intervals. Regressions between overall averages (of all ewes) of DFC and its STD in relation to the moving averages of weather variables were calculated for each season. Only where significant relationships occurred are these described or shown in tables and figures. The regressions were modelled in SAS 9.3 using the REG procedure.

2.2.3.2. Differences between-individual and population response

Using the analytical framework published by Nussey et al. (2007), random regression models were used to assess between-individual variation (random effects) and evaluate the population responses (fixed effects) to environmental parameters. Considering that the population-level response to the environment will depend on individual-level plasticity, the population response was modelled using the data from all animals and then the response from each animal (as a random effect) was compared with the population response and its deviation was calculated. The random regression models were done using the MIXED Procedure with the Restricted Maximum Likelihood (REML) method and COVTEST statement in SAS 9.3. Thus,

considering the relationship between trait y (in our case DFC for activity) and environmental variable e (in our case precipitation and/or temperature), both measured on occasion j , at individual level y_{ij} , the response of individual I on occasion j was specified as:

$$y_{ij} = \underbrace{\mu + \beta e_j}_{\text{Fixed effects}} + \underbrace{p_i + p_{ei}e_j + \varepsilon_{ij}}_{\text{Random effects}}$$

Where, μ = population mean on e ;

β = population mean slope of y on e .

p_i = deviation from the population average intercept (response that is independent of e , representing the individual elevation);

p_{ei} = deviation from the population average slope (response that is dependent of e , representing the individual plasticity).

e_j = Environmental variable;

ε_{ij} = residual error.

In each season, the ewes were divided into three groups based on the random regressions results: 1) fixed effects (data of all ewes modelling the population intercept and slope for weather variables and estimating the covariance parameter for intercept and slope(s)). A significant covariance parameter for the intercept implies that there were differences between individuals that were independent of the weather variables. A significant covariance parameter for the slope(s) for weather variables implies that there were differences between individuals in regard to the weather variables and; 2) random effects (data of all ewes modelling the deviation of each ewe from the population intercept and slope for weather variables). Thereafter, we will refer to the term individual phenotypic plasticity (as originally used by Nussey et al. (2007)), to support our division of individual sheep put into groups based upon the responses of their DFC data to the weather variables.

The three groups were: 1) Medium Consistency: represented by ewes that did not present a significant deviation from the population intercept and slope(s) ($P > 0.05$); 2) Low

Consistency: represented by ewes that negatively deviated from the population intercept or slope(s) ($P < 0.05$); and 3) High Consistency: represented by ewes that positively deviated from the population intercept or slope(s) ($P < 0.05$). The entire population could be classified in this way except one ewe, over the winter period. Data for this ewe, for winter only, deviated from the population intercept and slope, but exceptionally the deviations were in different directions, negatively deviated from the intercept (-11.0 ; $P < 0.001$) and positively deviated from the slope (0.74 ; $P < 0.03$). In this case, the higher and most significant deviation was used to determine in which group this animal would be and thus, over the winter period this ewe was included in the Low Consistency group. The best random regression models regarding the ewe responses to the weather were fitted for each season. Over the winter, the ewes were divided into these three groups considering the slope for precipitation (Supplementary Tables 2 and 3); over the spring, considering the slope for temperature (Supplementary Tables 5 and 6); over the summer (Supplementary Tables 8 and 9) and autumn (Supplementary Tables 11 and 12), considering the intercept.

After placing the ewes within their groups, the overall averages of BWG, DFC and STD of DFC for each group, in each season, were calculated. Data were checked for normal distribution using the PROC UNIVARIATE statement of SAS 9.3. Over the spring, summer and autumn the DFC and STD of DFC averages for the groups were not normally distributed; hence, angular transformations were used. To assist with interpretation of biological meaning, back-transformed means are presented in the results section. The effect of group and the effect of the presence of lambs (over spring and summer) were analysed using the MIXED Procedure of SAS 9.3 and the P values of the differences of least square means were adjusted for Tukey-Kramer. The significance level considered was 5%.

2.2.3.3. Links between production traits, general activity and circadian rhythm of activity

Regressions between BWG, motion index, STD of motion index, DFC and STD of DFC were calculated for each season, using the averages from all ewes. In addition, after the random regression analyses, the same regressions were done for each group within each season. Only

where significant and/or relevant relationships occurred are these described or shown in tables and figures. These regressions were modelled in SAS 9.3 using the REG procedure.

3. Results

Overall averages of BWG, motion index, STD of motion index, DFC% for activity and STD of DFC% for each season are shown in Table 1. The overall average of motion index was lower during the autumn compared with the spring. The DFC% for activity was highest and had the highest consistency (as shown by the STD of DFC%) during the autumn whereas it was lowest, with the lowest consistency during the winter. Over the winter, the ewes faced harsh weather that was linked to a breakdown in the circadian rhythm for activity and lowest BWG compared to the other seasons.

The general activity patterns of ewes during 24-hour changed over the year, corresponding to different daylight lengths observed between the seasons. Over the winter period, the day length was short and ewes showed activity bouts during both the daylight and dark phases. The general activity of ewes was higher during the daylight, with several activity peaks. During the dark phase, two resting bouts occurred, interrupted by one shorter activity bout with lower intensity (Figure 1, a). The length of the daylight period was longer during the spring and the general activity of ewes was consistently found only during the daylight with no activity bout during the dark phase. Over the spring period, there were several activity peaks, all during daylight (Figure 1, b). Similarly, the daylight period was very long during the summer and the general activity of ewes was higher during daylight, with several activity peaks and there was no activity bout during the dark phase (Figure 1, c). Over the autumn period, when the daylight began to shorten, ewes presented a pattern similar to the winter period (Figure 1, d).

As there were large differences between seasons regarding the general activity patterns and DFC% for activity, the results are displayed for each of the seasons separately. Given that the circadian rhythm of each animal is unique it is important to look at the detailed DFC%

response to understand whether it could be linked to production traits of each animal. Only when significant relationships occurred are these described or shown in tables and figures.

3.1. Winter

The ewes experienced a severe and challenging change in weather as the precipitation level reached up to 39 mm per day. The general activity of ewes expressed as motion index decreased showing a linear response in relation to precipitation (Figure 2a). The STD of the motion index also showed a linear reduction in response to increased precipitation (Figure 2b). However, the r-squares of these relationships were poor. The overall daily average of DFC% for activity presented a strong linear regression with the moving averages for precipitation (Figure 2c). Accordingly, the DFC% of the ewes decreased with increasing precipitation, descending to lower values with higher levels of precipitation. In addition, the variation of DFC% increased along with the precipitation increase, followed by a plateau, with a quadratic relationship providing the best relationship, suggesting the beginning of an adaptation pattern (Figure 2d).

The DFC% values of most ewes started to drop dramatically from the 13th and 14th days (Figure 3, b and c) when the precipitation level started to increase (Figure 3a). The differences between animals over the winter were mostly in regard to their different slope for precipitation (Supplementary Tables 2 and 3). For ewes in the Medium Consistency group (did not deviate from the population intercept and slope for precipitation) the DFC% continued to decrease until the 17th day and from this day started to show an adaptation pattern represented by increasing of DFC% levels, even with high levels of precipitation. Ewes in the High Consistency group presented a reduced variation of the DFC% response. In the Low Consistency group, ewes showed a greater decrease in the DFC% response; thereafter, the DFC% level of two ewes started to rise, but the ewe W134 did not show a recovering DFC% and died on the 20th day. Ewe G58 in the Low Consistency group showed deviation from the population slope and intercept. The positive deviation of the slope for precipitation showed that this ewe had a good capacity to cope with the precipitation level, however, it had a high negative deviation from the population intercept. Overall averages within groups are shown in Table 2.

Over the winter period, the population, in general, showed reductions in the DFC% response, as well as low ranges of BWG. However, there were no significant regressions between BWG and DFC% for activity (Supplementary Table 4). Considering the entire population (all ewes), there was no significant regression between BWG and motion index.

3.2. Spring

The between-individual variation of the DFC% response was well explained by the weather variables (Figure 4 and detailed statistical results in Supplementary Tables 5 and 6). Three ewes deviated from the population quadratic slope for precipitation and nine ewes deviated from the population slope for temperature, suggesting that temperature was the main effect for between-individual variation in the DFC% responses.

Regressions between BWG, DFC%, STD of DFC% and the presence and number of lambs are shown in Supplementary Table 7. Considering the entire population (all ewes), there was a significant negative multiple regression for BWG with STD of DFC% and the number of lambs; and a negative linear regression between STD of DFC% and BWG (Figure 5a). To gain a better understanding of this relationship and study the between-individual differences in the DFC% responses, linear regressions are also displayed within groups. The BWG of ewes in the Medium Consistency group was linked to lamb effect, with BWG decreasing with the increasing number of lambs. The BWG of ewes in the Low Consistency group was linked to the variation in the DFC% response, as there was a strong negative relationship between BWG and STD of DFC%, with BWG decreasing with the increasing STD of DFC% (Figure 5b). In addition, the number of lambs was not related to the high variation in the DFC% response shown by ewes in the Low Consistency group. Within the High Consistency group, the between-individual variation in the DFC% and its STD seems to be linked to the presence and number of lambs (Supplementary Figure 2). Considering the entire population (all ewes), there was no significant regression between BWG and motion index.

3.3. Summer

The regressions between the overall daily averages of DFC% for activity parameters and weather variables are shown in Supplementary Figure 3 and 4. Besides, these relations between the population DFC% and weather variables, the differences between-individual in the DFC% responses were regarding unknown factors that were independent of weather (Figure 6 and detailed statistical results in Supplementary Tables 8 and 9).

DFC% for activity was related to ewe performance; the regressions between BWG, number of lambs, DFC% and STD of DFC% considering all ewes and within groups are displayed in Supplementary Table 10. Considering all ewes, there was a negative linear relationship between BWG and STD of DFC%, indicating that ewes with lower variation in the DFC% response showed higher BWG (Figure 7a). The same negative linear relationship was present within the Medium Consistency group, suggesting that there is a close relationship between BWG and STD of DFC% (Figure 7b). In addition, considering all ewes, the DFC% and STD of DFC% were well explained by the BWG and number of lambs. Regarding the Low Consistency group, just two ewes negatively deviated over the summer period. Therefore, it was not possible to conduct a regression analysis, but it is relevant to mention that the ewe OR227, with the highest breakdown in activity rhythmicity, had the lowest BWG of the flock (-0.114 kg/day). Considering the entire population (all ewes), there was no significant regression between BWG and the motion index.

3.4. Autumn

The overall daily averages of DFC% for activity parameters showed a quadratic regression with the moving average of temperature (Supplementary Figure 5). Likewise in the summer period, over the autumn there were no differences between individual responses with temperature; and these differences between individuals were regarding an unknown factor that occurred within each animal (Figure 8 and detailed statistical results in Supplementary Tables 11 and 12). The ewe OR227 in the Low Consistency group repeatedly showed a high breakdown in the DFC% response, reaching low values. During the autumn period, there were no significant regressions between BWG and DFC% for activity (Supplementary Table 13).

Considering the entire population (all ewes), there were no significant regression between BWG and the motion index.

4. Discussion

The general activity patterns of ewes during 24-hours were different between seasons and this variation is believed to be mainly in response to the daylight length. Diurnal animals exhibit higher activity during daylight and the activity pattern is often related to feeding, especially for herbivorous animals (Bloch et al., 2013; Piccione et al., 2010; Umstätter et al., 2008). Umstätter et al. (2008) observed that extensively managed ewes in Scotland were active for over 60.5% of the daytime and spent 59.9% of the active time grazing and only 0.6% walking, without grazing. Thus, Umstätter et al. (2008) stated that based on activity data it is possible to define two behaviour categories, active (mainly grazing) and inactive (mainly recumbent).

Our study recorded additional activity after midnight with decreasing day length in autumn and winter. Langbein et al. (1996) also registered high proportions of nocturnal activity with decreasing day length in autumn and Champion et al. (1994) observed an eating bout over midnight. Considering that most activity behaviour is linked with feeding, it is reasonable to presume that ewes in our study spent time grazing overnight during the autumn and winter period. Longer periods of daylight, as observed during the spring and summer, are believed to allow adequate time for grazing. Accordingly, days with shorter daylight lengths, such as observed over the winter and autumn recording periods, were probably not enough to allow sufficient grazing, and associated rumination time, to satisfy nutritional needs and, thus, some grazing overnight was required.

Motion index data is thus important to understand the daily oscillation of activity behaviour and its variation between seasons, however, our results showed that activity behaviour alone had very poor or non-significant regression with the weather variables. In addition, we further analysed the motion index data using random regression models to understand the differences between animals regarding activity behaviour in response to the

weather variables. However, the weather had little or no effect on the between-individual differences and over all seasons these differences were regarding the individual reaction norm elevation (Nussey et al., 2007). Our results showed that examining data in a different way, by analysing the circadian rhythm through the calculation of DFC% for activity and its variation (STD of DFC%) enables a better understanding of animal responses with respect to the weather influences compared to using motion index data alone. In addition, the random regression model method was effective in identifying animals whose DFC% deviated from population responses. Considering the new proposal of this study, a deviation from the DFC% intercept is a way of measuring a temporary distress within the individual. Further, this methodology also linked this disturbance to weather events and changes, when some animals deviated from the population slope for weather variables.

In this study, the weather variables influenced the activity rhythmicity of ewes over all seasons. But, the links between weather and rhythmicity were different between seasons. Over the winter period, the largest decrease in the DFC% response of ewes was linked with high precipitation level, during which weather events there was also lower temperatures. The quadratic response between DFC% in relation to moving average of daily precipitation over the spring period suggest that sheep were less affected by rain when the temperatures were higher in spring. Our result is in line with Warren and Mysterud (1991) who studied sheep on a coniferous forest range in southern Norway and observed that sheep activity patterns were rhythmic and consistent throughout the summer season, although were affected by both weather conditions and day length. These authors also observed that cold ($< 10^{\circ}\text{C}$), wet weather or foggy conditions reduced the overall activity of the flock. Other studies of extensively managed sheep have shown that they reduce grazing during heavy winter rainfall, but are less affected by rain in the summer months (Champion et al., 1994; Hunter, 1995).

Abrupt environmental changes, such as periods of high precipitation levels with low temperatures faced by ewes over the winter, may have a direct effect on the activity rhythmicity. This lack of rhythmicity may persist until the end of the environmental disturbance, the animal

adaptation or its failure to adapt adequately. The animal failure may result in poor welfare and potentially death. However, the degree of these negative effects can clearly differ between individuals and we postulate that this difference is an outcome of the animal's ability to cope with short-term environmental change, its individual phenotypic plasticity (deviation from the population slope for the weather variable). Analysing the individual DFC response combined with the use of random regression models to classify the differences between each animal's ways of coping with the challenging environment was a novel approach of our study. We believe this has proved to be successful.

Knowledge of individual plasticity is important because the population level response to the environment will depend on individual levels of phenotypic plasticity (Dingemanse et al., 2010; Nussey et al., 2007; Wilson, 1998). We found weather variables influenced ewe DFCs for activity over all seasons. However, only during the winter and spring was there the association between the weather and the variation between animals. During the summer and autumn periods, between-individual variation in DFC% data was not related to temperature or precipitation. These findings suggest that the inconsistent rhythmicity of ewes in the Low Consistency group over the summer and autumn periods were linked with others factors that we did not measure. However, it can be assumed that these were particular factors within these animals.

When animals are experiencing an environmental disturbance, we typically found the DFC% for activity is low. Over the winter period, all ewes had breakdowns in the circadian rhythm of activity, even ewes in the High Consistency group. However, the ewes in the High Consistency group showed less variation of the DFC% response, evidencing that their plastic response was positive and enabled them to cope better with higher levels of precipitation and consequently showing a faster adaptation. On the other hand, ewes in the Low Consistency group showed a stronger decrease in DFC% reaching values lower than 30%, as well as a more difficult recovery of the DFC% response, suggesting an adaptation problem. Some of these ewes had a maladaptive plasticity, with a greater lack of circadian rhythmicity and an indication

of poorer welfare, and consequently a great risk of poor health and death. A lack of circadian rhythmicity caused by an environmental change might be temporary and not affect animal health, however, it also might be harmful, as the long-term circadian rhythm disorganization acts as stress factor that may have consequences to animal health. Dwyer and Bornett (2004) noted that chronic stress induces alterations in behaviour and its circadian rhythms, particularly for activity and feeding. These authors also observed that chronic stress reduce immune function. Martino et al. (2008) have noted that circadian organization is critical for normal health and longevity, whereas circadian rhythm disorganization produces profound cardiovascular and renal disease.

The winter recording period had the highest level of nocturnal activity after midnight, the lowest overall DFC%, the greatest overall variation through STD of DFC%, and a clear reduction in DFC% linked to high rainfall and cold temperature. The Low Consistency group during this period had a high level of mortality. Ewe mortality and welfare are seen as industry-wide issues during this period (Morgan-Davies et al., 2008). The highest level of nocturnal activity after midnight over the winter was probably an attempt to obtain heat rewards, by feeding. Shorter daylight lengths were probably not enough to allow sufficient grazing, and associated rumination time, to satisfy nutritional needs. When energy intake is restricted, metabolic rate is correspondingly reduced to correct for the energy imbalance, a process called diet-induced thermogenesis (Stock, 1999). The reduction in metabolic rate is accompanied by a lowering of body temperature and this is observed primarily during the inactive phase (Stock, 1999). Thus, while exposed to a cold environment, animals would try to obtain heat rewards, by feeding overnight, to suppress lowering of body temperature attempting to regulate body temperature at the normothermic level (Piccione et al., 2002). In addition, the energy costs of cold exposure must eventually be reflected in poor animal performance (Slee, 1971), as observed in this study over the winter period. However, this does not demonstrate the ewes were in thermal distress but it may be an attempt to prevent it.

In a review, Dwyer and Bornett (2004) state that in hot weather, sheep adjust their diurnal patterns to the coolest times of the day, but when temperatures are low, wind speed is high and/or it is raining, sheep make use of shelter; a mechanism that help animals to deal with thermal extremes and decrease thermoregulation risk. However, these authors note that cold-exposed sheep, but lacking in shelter, may experience thermal distress. According to Nikkhah (2012), eating time is a major external cue and a feasible life strategy that affects production and health physiology. In the Low Consistency group, over the winter, ewes showed a greater decrease in the DFC% response. Thereafter, the DFC% level of two ewes started to rise but ewe W134 did not show a recovering DFC% and died on the 20th day. DFC% combined with random regression model appeared to predict the death of this ewe and showed the link with the harsh weather. Ewe G58 in the Low Consistency group showed deviation from the population slope and intercept. The positive deviation of the slope for precipitation showed that this ewe had a good capacity to cope with the precipitation level, however, it had a high negative deviation from the population intercept. DFC% combined with random regression model detected that its death was linked to a factor that was independent of the weather and occurred within this animal. The death of this ewe was regarded as an accident as it caught its leg in the feeding ring. Ewe B252 in the Low Consistency group also died a few days after the end of measurements. Looking at the ewe mortality in the Low Consistency group, three of four ewes in this group died over the winter period, while there were no deaths for the other 19 ewes in the Medium and High Consistency groups. The probability of the actual distribution of deaths/survivals amongst Low and combined Medium/High Consistency groups is $P = 0.002$. Thus, the probability of death in the Low Consistency group is higher than by chance alone further indicating that this approach may have a value for monitoring livestock in extensive systems. The flock from which this study flock was taken had a study conducted in Morgan-Davies et al. (2008) when the annual ewe mortality was reported as 8.2%, similar to that found in our study group. These levels of mortality highlight the need for improved husbandry, where new technology may play a part.

Berger et al. (1999) observed similar results to those in our study, in research with Przewalski horse. Przewalski horses showed lowered DFCs in periods of adaptation after translocation from a zoo to a semi-reserve. Lowered DFCs of Przewalski horse were also observed during the period of hunting and shooting in the surrounding area of the semi-reserve (Scheibe et al., 1999). These findings show that not only weather variables can cause circadian rhythm disorganization, but also other stressful environmental disturbances. Thus, other environmental data sets with daily observations could potentially be used to evaluate the differences between individual rhythmicity and the way that animals cope with their environment. Moreover, health problems, injury, immobilization, social interactions and even some normal physiological changes such as parturition may also be the cause of a lack of behavioural circadian rhythmicity (Berger et al., 2003, 1999; Scheibe et al., 1999, 1998). Our study has confirmed the potential value of the chronobiological approach to analysing activity behaviour.

The relationship between BWG and the variation of DFC% for activity was noteworthy over the spring and summer periods. Over the spring period, the BWG of the flock was well explained by a combination of presence and number of lambs and by the variation of DFC% for activity. However, clustering ewes into groups allowed a better understanding of these relationships. The BWG of the ewes in the Medium Consistency group showed a significant regression with the number of lambs, with an r-square similar to the entire flock regression. Thus, the influence of the lambs for the entire flock was mostly driven by the ewes in the Medium Consistency group. It can be assumed that the differences between-individual BWG for the Medium Consistency group was related to the metabolic rate of the ewes. The spring period measurements occurred soon after parturition and ewes with lambs were in early lactation, and under higher metabolic demand for milk production, and thus showing lower BWG. Likewise, the influence of the variation of the DFC% for the entire flock was mostly driven by the ewes in the Low Consistency group. The BWG of the ewes in the Low Consistency group showed a significant regression with the STD of DFC%, with a higher r-square than the one found for the

590 regression considering the entire flock. Thus, the differences between-individual BWG for the
 591 Low Consistency group was related only to the variation of the DFC%.

592 During the summer period, there were no significant regressions between BWG and
 593 number and presence of lambs. Ewes with lambs were in late lactation, with reduced milk yield
 594 and then BWG will be less directly influenced by the presence and number of lambs.
 595 Nevertheless, BWG was associated with the variation of DFC% for activity. Ewes with a lower
 596 variation of DFC% showed higher BWG. Regarding the Low Consistency group, just two ewes
 597 negatively deviated over the summer period. Therefore, it was not possible to display a
 598 regression analysis, but it is important to mention that the ewe OR227, with the highest
 599 breakdown in activity rhythmicity, showed the lowest BWG of the flock (-0.114 kg/day).
 600 Autumn measurements were made after weaning, a period typical of rapid weight gain with no
 601 reproduction demand and it is clear to see that almost all ewes were consistent in the DFC%
 602 response. Considering that motion index had no significant regression with BWG or presence of
 603 lambs, the variation in the DFC% response was a better estimator of BWG than the use of
 604 simple motion index.

605 Analysing the DFC% for activity and the differences between individuals using the
 606 random regression model enabled the detection not only of environmental disturbances but
 607 showed that it is also possible to detect animals with particular issues. In addition, clustering
 608 ewes into groups using random regression models allowed a better understanding of the
 609 relationships between BWG, the presence of lambs and STD of DFC% over the spring period.
 610 The new framework proposed by this study, combining DFC analyses and random regression
 611 model could be used in practice to understand whether the differences between animals were in
 612 response to the environment or whether the difference was shown in relation to a particular
 613 problem by a particular animal linked to animal performance. Further, the measurement of
 614 individual DFCs may have potential to help the genetic selection of healthy and adapted animals
 615 and to provide a monitoring tool for health and welfare issues.

The current study was conducted with raw data that was created and saved on a memory card, and post-processed. With further technological developments, animal-based sensors may increasingly be part of a real-time decision support system for researchers, veterinarians or farmers with circadian rhythm analysis providing a different perspective to that from the only short-term, real-time visualisation of data. Additionally, studies of circadian rhythmicity could be a useful contributor to the better understanding of biology and to evaluate animal husbandry systems considering aspects of animal welfare.

5. Conclusions

This study confirmed the strong dynamic created by the seasons and by the production/physiological cycle in sheep in high latitude systems. The analysis of the circadian rhythm of activity using the DFC-parameter enables a better understanding of sheep responses to weather and environmental influences, compared to the use of a simple quantitative activity parameter, such as motion index. The random regression model method was effective in identifying animals that deviated from population responses regarding the weather influence or to a particular problem. Over the spring and summer periods, the variation in the DFC response was a better estimator of BWG than the use of a simple motion index. Clustering ewes into groups using random regression models allowed a better understanding of the relationships between BWG, the presence of lambs and STD of DFC% over the spring period.

The combination of circadian rhythm analysis and the clustering of individuals into groups based on their regression response to environmental variables provides a considerable potential to glean information relevant to group and individual animal management. With increasing availability of such data captured through automated telemetric systems, this work shows that these approaches may enhance the quality and meaningfulness of data coming from automated sensors.

6. Acknowledgement

SRUC receives financial support from the Scottish Government. CAPES Foundation, Ministry of Education of Brazil supported the scholarship – Process 99999.004594/2014-02.

7. References

- Andel, J., 1984. Statistische Analyse von Zeitreihen, Berlin: Akademie-Verlag.
- Berger, A., Scheibe, K.-M., Michaelis, S., Streich, W.J., 2003. Evaluation of living conditions of free-ranging animals by automated chronobiological analysis of behavior. *Behav. Res. Methods, Instruments, Comput.* 35, 458–466.
doi:<https://doi.org/10.3758/BF03195524>
- Berger, A., Scheibe, K.M., Eichhorn, K., Scheibe, A., Streich, J., 1999. Diurnal and ultradian rhythms of behaviour in a mare group of Przewalski horse (*Equus ferus przewalskii*), measured through one year under semi-reserve conditions. *Appl. Anim. Behav. Sci.* 64, 1–17. doi:[10.1016/S0168-1591\(99\)00026-X](https://doi.org/10.1016/S0168-1591(99)00026-X)
- Bloch, G., Barnes, B.M., Gerkema, M.P., Helm, B., 2013. Animal activity around the clock with no overt circadian rhythms: patterns, mechanisms and adaptive value. *Proc. R. Soc. B Biol. Sci.* 280, 20130019–20130019. doi:[10.1098/rspb.2013.0019](https://doi.org/10.1098/rspb.2013.0019)
- Champion, R.A., Rutter, S.M., Penning, P.D., Rook, A.J., 1994. Temporal variation in grazing behaviour of sheep and the reliability of sampling periods. *Appl. Anim. Behav. Sci.* 42, 99–108. doi:[10.1016/0168-1591\(94\)90150-3](https://doi.org/10.1016/0168-1591(94)90150-3)
- Dingemanse, N.J., Kazem, A.J.N., Réale, D., Wright, J., 2010. Behavioural reaction norms: animal personality meets individual plasticity. *Trends Ecol. Evol.* 25, 81–89. doi:[10.1016/j.tree.2009.07.013](https://doi.org/10.1016/j.tree.2009.07.013)
- Dingemanse, N.J., Wolf, M., 2013. Between-individual differences in behavioural plasticity within populations: causes and consequences. *Anim. Behav.* 85, 1031–

- 664 1039. doi:10.1016/j.anbehav.2012.12.032
- 665 Dwyer, C., Bornett, H., 2004. Chronic stress in sheep: assessment tools and their use in
666 different management conditions. *Anim. Welf.* 13, 293–304.
- 667 Firk, R., Stamer, E., Junge, W., Krieter, J., 2002. Automation of oestrus detection in
668 dairy cows: A review. *Livest. Prod. Sci.* 75, 219–232. doi:10.1016/S0301-
669 6226(01)00323-2
- 670 Foster, R.G., Kreitzman, L., 2005. Rhythms of life: the biological clocks that control the
671 daily lives of every living thing. Yale University Press.
- 672 Herczeg, G., Garamszegi, L.Z., 2012. Individual deviation from behavioural
673 correlations: A simple approach to study the evolution of behavioural syndromes.
674 *Behav. Ecol. Sociobiol.* 66, 161–169. doi:10.1007/s00265-011-1291-2
- 675 Hunter, R.F., 1995. Some notes on the behaviour of hill sheep. *Br. J. Anim. Behav.* 2,
676 75–78. doi:10.1016/S0950-5601(54)80036-0
- 677 Koukkari, W.L., Sothorn, R.B., 2006. The Study of Biological Rhythms, Introducing
678 Biological Rhythms. doi:10.1007/978-1-4020-4701-5_1
- 679 Langbein, J., Scheibe, K.M., Eichhorn, K., Lindner, U., Streich, W.J., 1996. An
680 activity-data-logger for monitoring free-ranging animals. *Appl. Anim. Behav. Sci.*
681 48, 115–124. doi:10.1016/0168-1591(95)01018-1
- 682 Lincoln, G.A., Lincoln, C.E., Mcneilly, A.S., 1990. Seasonal cycles in the blood plasma
683 concentration of FSH, inhibin and testosterone, and testicular size in rams of wild,
684 feral and domesticated breeds of sheep. *J. Reprod. Fertil.* 88, 623–633.
685 doi:http://dx.doi.org/10.1530/jrf.0.0880623
- 686 Lincoln, G.A., Richardson, M., 1998. Photo-neuroendocrine control of seasonal cycles

- 687 in body weight, pelage growth and reproduction: lessons from the HPD sheep
688 model. *Comp. Biochem. Physiol. Part C* 119, 283–294.
- 689 Martino, T.A., Oudit, G.Y., Herzenberg, A.M., Tata, N., Koletar, M.M., Kabir, G.M.,
690 Belsham, D.D., Backx, P.H., Ralph, M.R., Sole, M.J., 2008. Circadian rhythm
691 disorganization produces profound cardiovascular and renal disease in hamsters.
692 *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 294, R1675-83.
693 doi:10.1152/ajpregu.00829.2007
- 694 Morgan-Davies, C., Waterhouse, A., Pollock, M.L., Milner, J.M., 2008. Body condition
695 score as an indicator of ewe survival under extensive conditions. *Anim. Welf.* 17,
696 71–77.
- 697 Nikkhah, A., 2012. Eating time modulations of physiology and health: Life lessons
698 from human and ruminant models. *Iran. J. Basic Med. Sci.* 15, 891–899.
- 699 Nussey, D.H., Wilson, A.J., Brommer, J.E., 2007. The evolutionary ecology of
700 individual phenotypic plasticity in wild populations. *J. Evol. Biol.* 20, 831–44.
701 doi:10.1111/j.1420-9101.2007.01300.x
- 702 Piccione, G., Caola, G., Refinetti, R., 2005. Temporal relationships of 21 physiological
703 variables in horse and sheep. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.*
704 142, 389–96. doi:10.1016/j.cbpa.2005.07.019
- 705 Piccione, G., Caola, G., Refinetti, R., 2002. Circadian modulation of starvation-induced
706 hypothermia in sheep and goats. *Chronobiol. Int.* 19, 531–41.
707 doi:https://doi.org/10.1081/CBI-120004225
- 708 Piccione, G., Giannetto, C., Marafioti, S., Casella, S., Caola, G., 2010. The effect of
709 photic entrainment and restricted feeding on food anticipatory activity in *Ovis*

- 710 aries. *Small Rumin. Res.* 94, 190–195. doi:10.1016/j.smallrumres.2010.07.017
- 711 Pigliucci, M., 2001. *Phenotypic Plasticity: Beyond Nature and Nurture*. JHU Press.
- 712 doi:doi:10.1038/sj.hdy.6800153
- 713 Refinetti, R., Menaker, M., 1992. The circadian rhythm of body temperature. *Physiol.*
- 714 *Behav.* 51, 613–37. doi:DOI: 10.3758/BF03203466
- 715 Scheibe, K.M., Berger, a., Langbein, J., Streich, W.J., Eichhorn, K., 1999. *Comparative*
- 716 *Analysis of Ultradian and Circadian Behavioural Rhythms for Diagnosis of*
- 717 *Biorhythmic State of Animals.* *Biol. Rhythm Res.* 30, 216–233.
- 718 doi:10.1076/brhm.30.2.216.1420
- 719 Scheibe, K.M., Schleusner, T., Berger, a., Eichhorn, K., Langbein, J., Dal Zotto, L.,
- 720 Streich, W.J., 1998. ETHOSYS (R)—new system for recording and analysis of
- 721 behaviour of free-ranging domestic animals and wildlife. *Appl. Anim. Behav. Sci.*
- 722 55, 195–211. doi:10.1016/S0168-1591(97)00072-5
- 723 Sih, A., Bell, A., Johnson, J.C., 2004. Behavioral syndromes: an ecological and
- 724 evolutionary overview. *Trends Ecol. Evol.* 19, 372–8.
- 725 doi:10.1016/j.tree.2004.04.009
- 726 SLEE, J., 1971. Physiological factors affecting the energy cost of cold exposures, in:
- 727 *Proceedings of the Nutrition Society.* pp. 215–221.
- 728 Stock, M.J., 1999. Gluttony and thermogenesis revisited. *Int. J. Obes. Relat. Metab.*
- 729 *Disord.* 23, 1105–17. doi:10.1038/sj.ijo.0801108
- 730 Umstätter, C., Waterhouse, A., Holland, J.P., 2008. An automated sensor-based method
- 731 of simple behavioural classification of sheep in extensive systems. *Comput.*
- 732 *Electron. Agric.* 64, 19–26. doi:10.1016/j.compag.2008.05.004

- 733 Warren, J.T., Mysterud, I., 1991. Summer habitat use and activity patterns of domestic
734 sheep in coniferous forest range in Southern Norway. *J. Range Manag.* 44, 2–6.
735 doi:DOI: 10.2307/4002628
- 736 Wilson, D.S., 1998. Adaptive individual differences within single populations. *Philos.*
737 *Trans. R. Soc. B Biol. Sci.* 353, 199–205. doi:10.1098/rstb.1998.0202
- 738 Wood, S., Loudon, A., 2014. Clocks for all seasons: unwinding the roles and
739 mechanisms of circadian and interval timers in the hypothalamus and pituitary. *J.*
740 *Endocrinol.* 222, R39-59. doi:10.1530/JOE-14-0141
- 741

Table 1 Body Weight Gain (BWG), Motion Index (MI), Degree of Functional Coupling (DFC) for activity, MI and DFC standard deviation (STD) for each season.

	Seasons				SEM ²	P ³
	Winter	Spring ¹	Summer ¹	Autumn		
	mid-	after	late	after		
	pregnancy	parturition	lactation	weaning		
BWG, kg/day	0.05 ^b	0.18 ^a	0.20 ^a	0.20 ^a	0.03	< 0.001
MI	3895 ^{ab}	4596 ^a	3422 ^{ab}	2907 ^b	374	< 0.01
STD of MI	1866 ^a	1122 ^b	818 ^{bc}	564 ^c	224	< .0001
DFC, %	81.66 ^c	88.06 ^{bc}	90.22 ^{ab}	95.38 ^a	1.65	< .0001
STD of DFC, %	17.39 ^a	9.77 ^b	8.90 ^{bc}	5.39 ^c	1.28	< .0001

¹ Fixed effect of lamb was not significant ($P > 0.05$). ² Maximum standard error of the mean. ³ P value for the fixed effect of season. ^{a,b,c} Means with different superscripts along the same line are significantly different with $P < 0.05$ for differences of least squares means.

Table 2 Overall averages of Degree of Functional Coupling (DFC) for activity, DFC standard deviation (STD) and Body Weight Gain (BWG) for each group within the seasons.

	Group			SEM ¹	P ²
	High	Medium	Low		
	Consistency	Consistency	Consistency		
Winter					
DFC%	93.1 ^a	82.7 ^b	66.5 ^c	3.32	< .0001
STD of DFC%	8.6 ^c	16.4 ^b	30.1 ^a	2.65	< 0.001
BWG, kg	0	0.08	-0.07	0.08	0.14
Spring ³					
DFC%	96.42 ^a	90.35 ^b	76.98 ^c	2.09	< .0001
STD of DFC%	5.69 ^b	8.43 ^b	14.29 ^a	1.49	< 0.001
BWG, kg	0.246	0.223	0.111	0.052	0.13
Summer ³					
DFC%	97.65 ^a	91.87 ^b	69.30 ^c	2.98	< .0001
STD of DFC%	4.81 ^c	8.50 ^b	22.00 ^a	2.82	< 0.01
BWG, kg	0.146	0.218	0.086	0.072	0.16
Autumn					
DFC%	99.84 ^a	96.52 ^b	87.56 ^c	0.78	< .0001
STD of DFC%	0.72 ^c	4.89 ^b	11.75 ^a	1.73	< .0001
BWG, kg	0.099	0.221	0.167	0.089	0.43

¹ Maximum standard error of the mean. ² P value for the fixed effect of season. ³ Fixed effect of lamb was not significant ($P > 0.05$). ^{a,b,c} Means with different superscripts along the same line are significantly different with $P < 0.05$ for differences of least squares means.

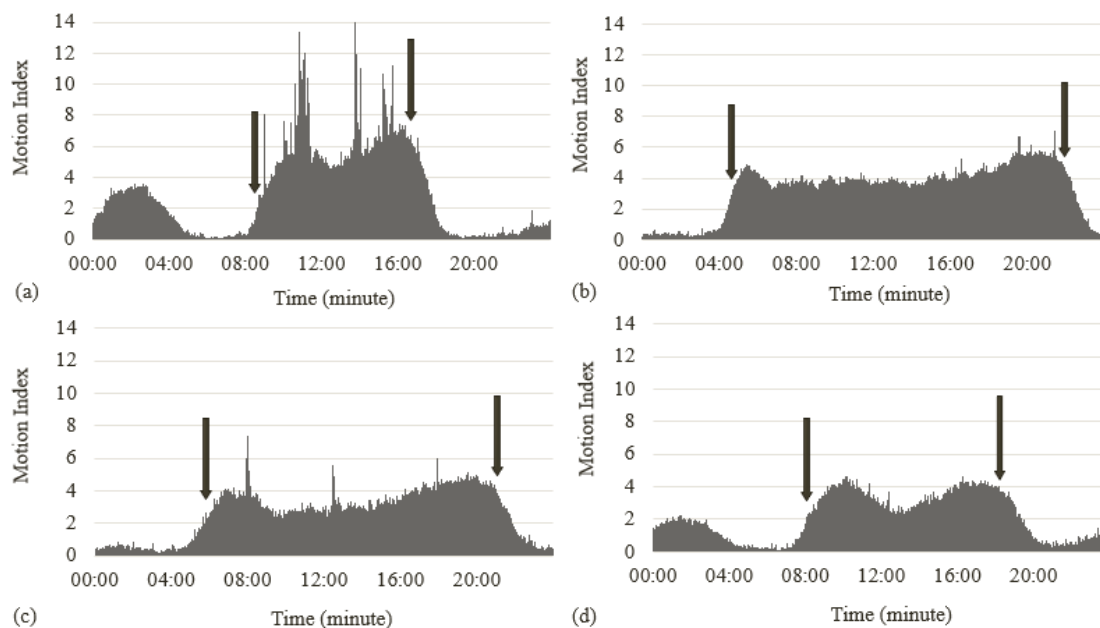


Figure 1. General activity patterns of ewes during 24-hour over the seasons. One bar per minute and every minute value is an overall average for motion index of all ewes. A low motion index corresponds to a low activity level and a high motion index corresponds to a high activity level. Arrows show average time of sunrise and sunset. (a) Winter period: Jan/Feb, 30 days, 23 ewes, sunrise was between 07:57 – 08:46 h and sunset between 16:08 – 17:09 h. (b) Spring period: May/Jun, 28 days, 24 ewes, sunrise was between 04:28 – 04:51 h and sunset between 21:39 – 22:11 h. (c) Summer period: August, 28 days, 19 ewes, sunrise was between 05:20 – 06:14 h and sunset between 20:26 – 21:30 h. (d) Autumn period: October, 26 days, 24 ewes, sunrise was between 07:30 – 08:22 h and sunset between 17:42 – 18:45 h.

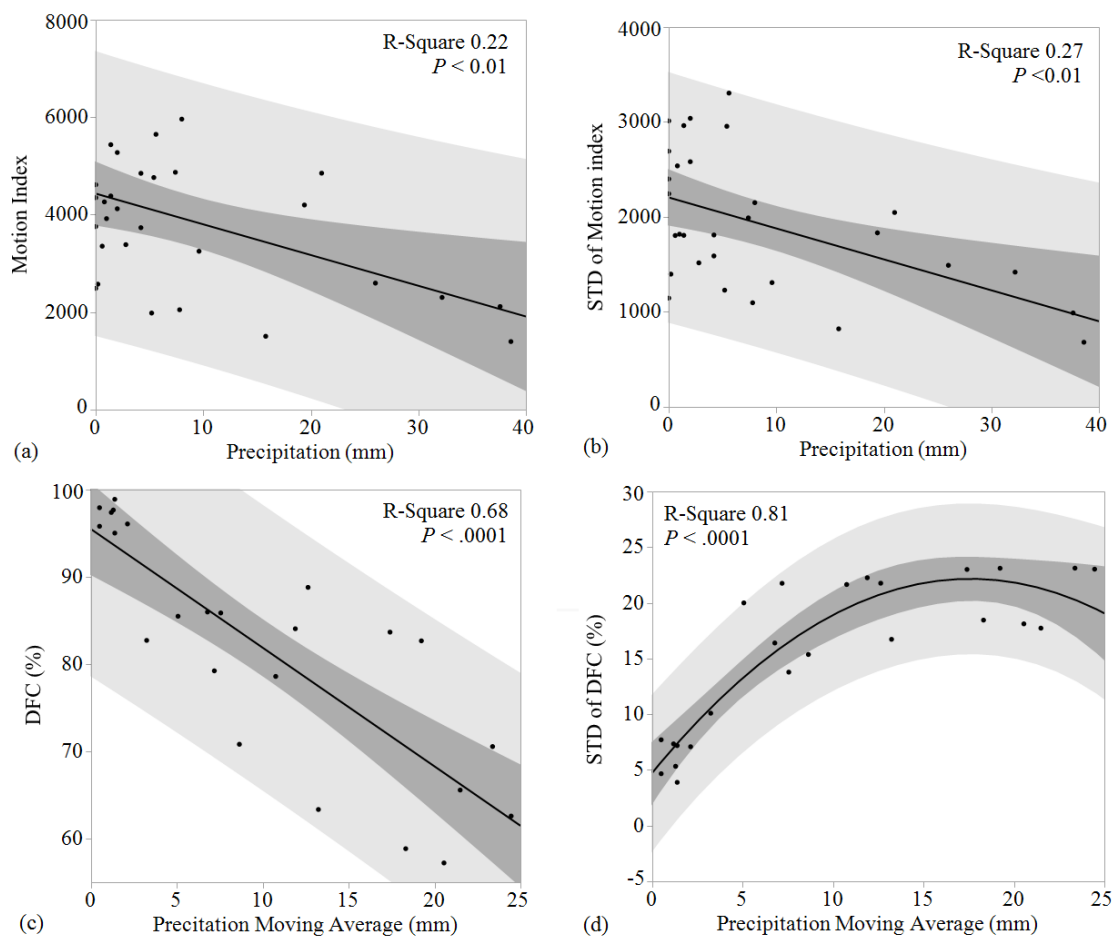


Figure 2. General activity (motion index) and Degree of Functional Coupling (DFC%) for the activity of ewes in response to precipitation level over the winter period. The dark grey area represents 95% confidence limits and the light grey area represents 95% prediction limits. (a) Linear regression between the overall daily average of motion index and daily precipitation. (b) Linear regression between the overall daily average of standard deviation (STD) of motion index and daily precipitation. (c) Linear regression between DFC% for activity and moving averages for precipitation (DFC% was calculated within moving seven-day intervals, thus, to allow juxtaposition, the moving average for precipitation was also set up in seven-day intervals. Each DFC% value is an overall average of all ewes). (d) Quadratic regression between the standard deviation of DFC% for activity and moving average for precipitation.

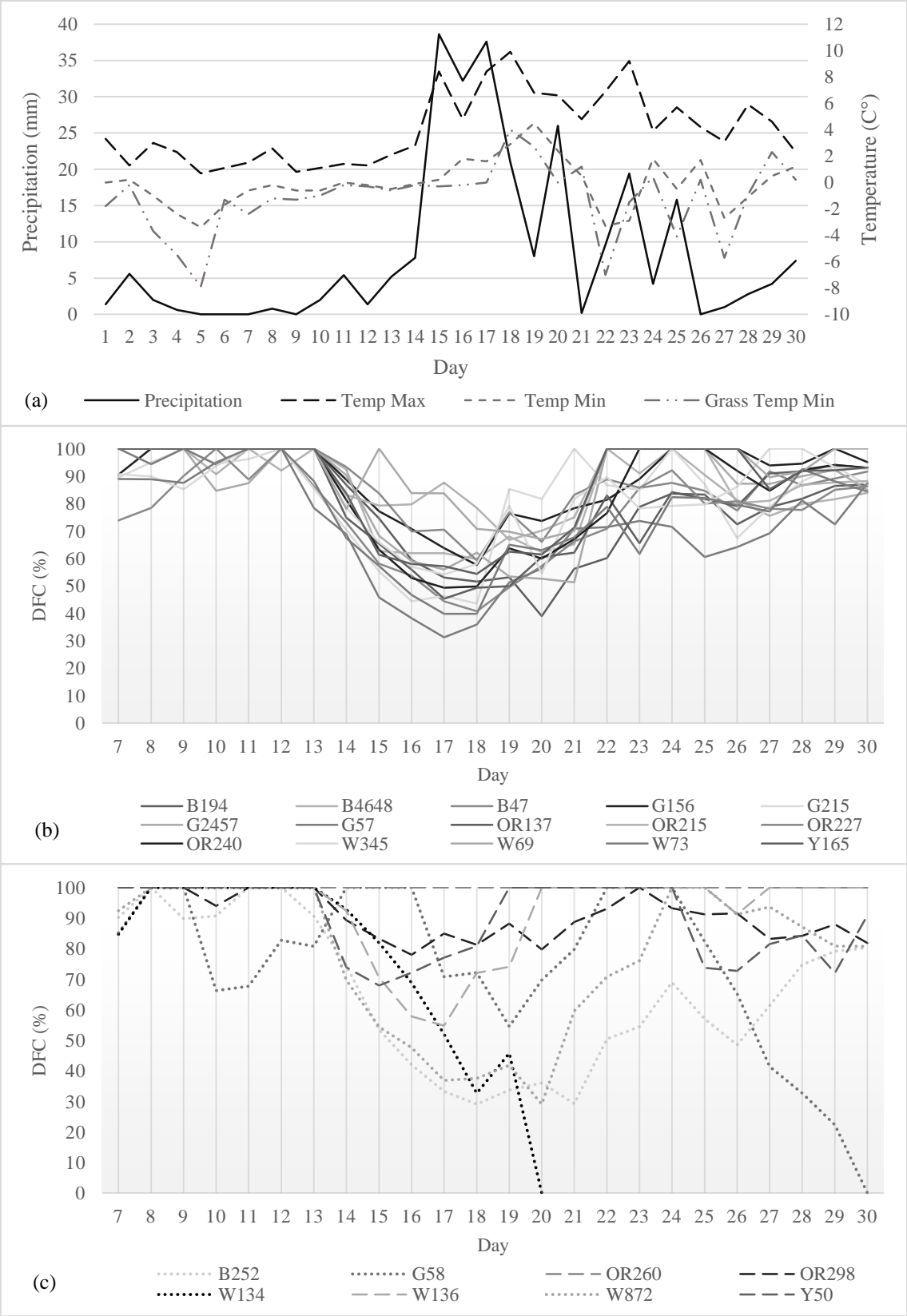


Figure 3. Degrees of Functional Coupling (DFCs) for the activity of ewes over the winter period. DFC% was calculated for moving seven-day intervals, thus, the graphic starts at the 7th day. (a) Absolute values for weather variables over the winter period. (b) DFC% response of 15 ewes that did not present deviation for weather variables over the winter period. (c) DFC% response of 15 ewes that did not present deviation for weather variables over the winter period.

779 from the population intercept and slope over the winter period (Medium Consistency group). DFC% of
780 these ewes declined until the 17th day and from this day started to show an adaptation pattern represented
781 by an increase of DFC% levels, even with high levels of precipitation. (c) DFC% response of eight ewes
782 that presented deviation from the population slope over the winter period. The four ewes represented by
783 dashed lines showed a reduced decrease of DFC% (High Consistency group) whereas the four ewes
784 represented by dotted lines showed a greater decrease of DFC% (Low Consistency group). The ewes
785 W134 and G58 died on 20th and 30th, respectively. The ewe G58 also showed deviation from the
786 population intercept.

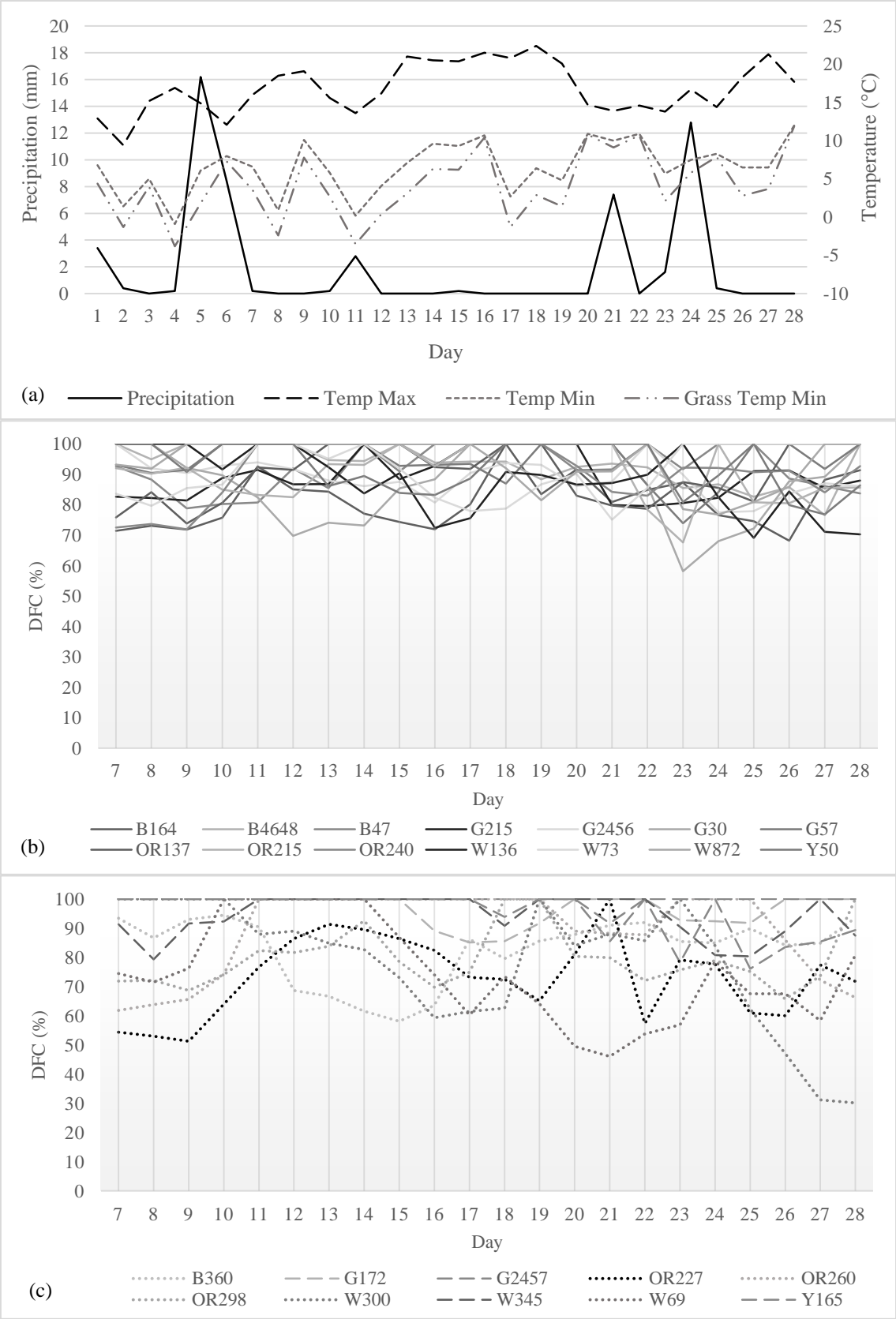


Figure 4. Degrees of Functional Coupling (DFCs) for the activity of ewes over the spring period. DFC% was calculated for moving seven-day intervals, thus, the graphic starts at the 7th day. (a) Absolute daily values for weather variables over the spring period. (b) DFC% response of 14 ewes that did not present values for weather variables over the spring period. (c) DFC% response of 14 ewes that did not present values for weather variables over the spring period.

deviation from the population intercept and slopes over the spring period (Medium Consistency group). (c) DFC% response of 10 ewes that presented deviation from the population temperature or squared precipitation slope over the spring period. Four ewes represented by dashed lines presented higher consistency of DFC% (High Consistency group), whereas six ewes represented by dotted lines showed the lower consistency of DFC% (Low Consistency group). Ewe OR260 showed deviation from the population slope for squared precipitation. Ewes B360 and OR227 showed deviation from both temperature and squared precipitation slopes. The others ewes showed deviation from the population slope for temperature.

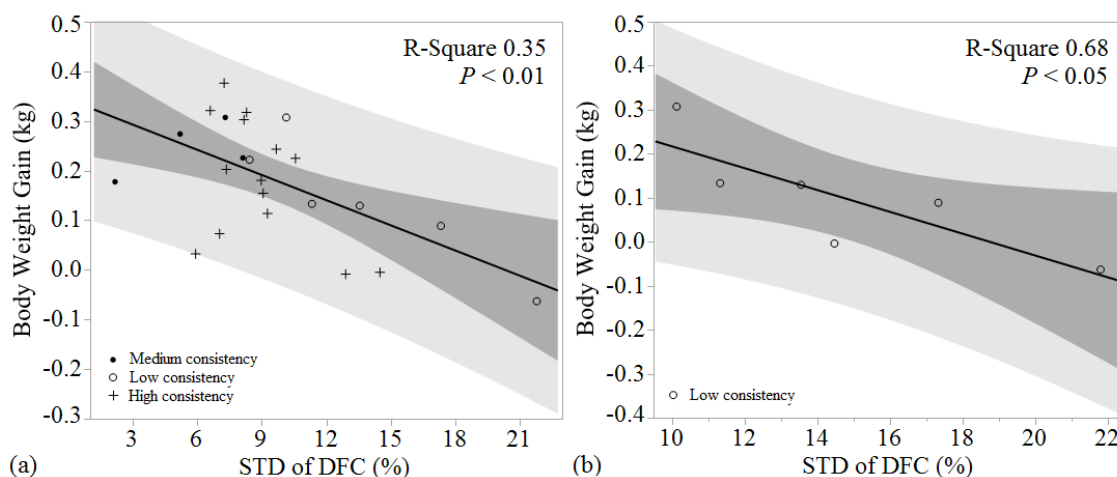


Figure 5. Body weight gain (BWG) in relation to the variation of the Degrees of Functional Coupling (DFC) for activity, over the spring period. The dark grey area represents 95% of confidence limits and the light grey area represents 95% of prediction limits. Each DFC% value is an overall average for each ewe, over the spring period. (a) Linear regression for BWG in relation to the standard deviation (STD) of DFC% for all ewes. (b) Linear regression for BWG in relation to STD of DFC% for the Low Consistency group.

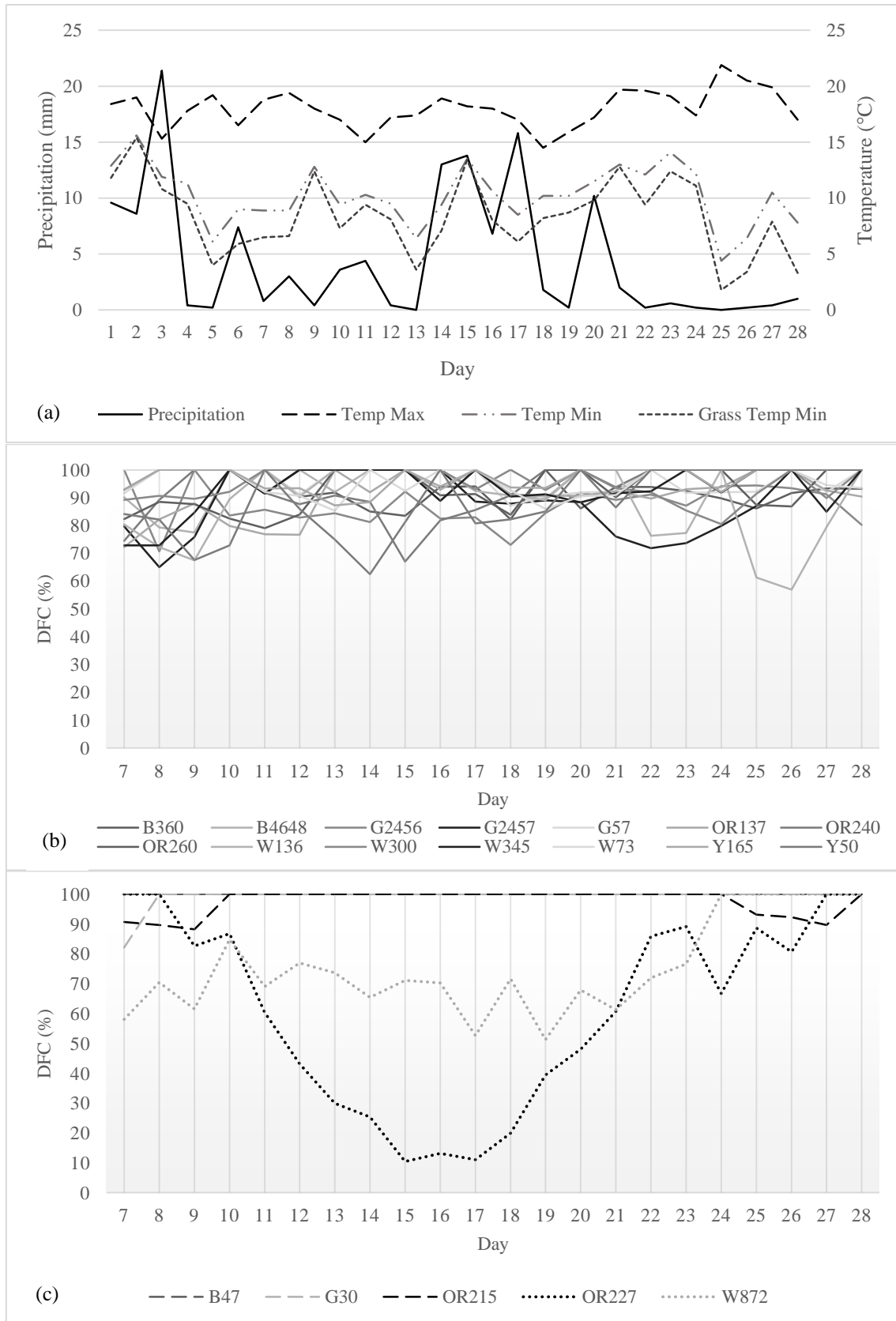


Figure 6. Degrees of Functional Coupling (DFCs) for the activity of ewes over the summer period. DFC% was calculated for moving seven-day intervals, thus, the graphic starts at the 7th day. (a) Absolute values for the weather over the summer period. (b) DFC% response of 14 ewes that did not present

deviation from the population intercept and slope over the summer period (Medium Consistency group). (c) DFC% response of five ewes that showed a deviation from the population intercept over the summer period. The three ewes represented by dashed lines presented higher consistency of DFC% (High Consistency group), whereas the two ewes represented by dotted lines showed the lower consistency of DFC% (Low Consistency group).

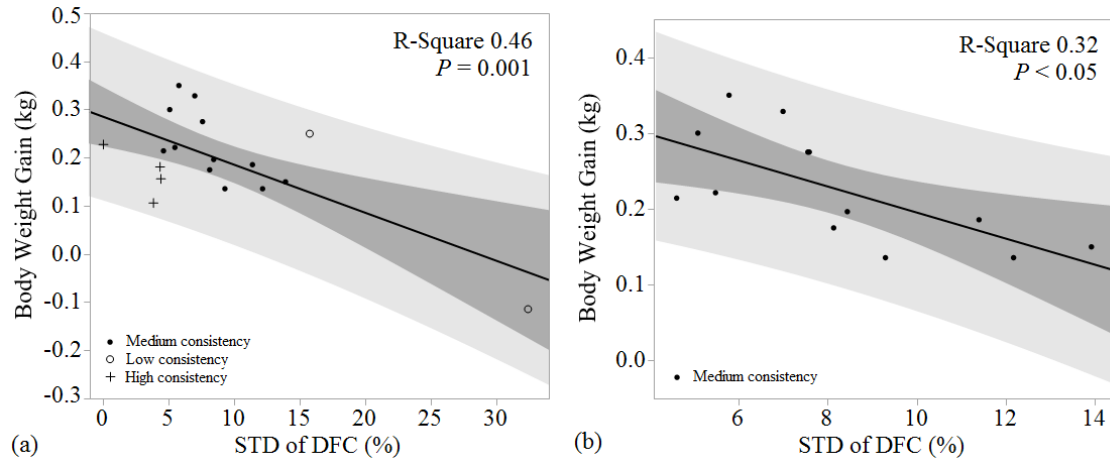


Figure 7. Body weight gain (BWG) in relation to the variation of the Degrees of Functional Coupling (DFC) for activity, over the summer period. The dark grey area represents 95% of confidence limits and the light grey area represents 95% of prediction limits. Each DFC% value is an overall average for each ewe, over the spring period. (a) Linear regression for BWG in relation to the standard deviation (STD) of DFC% for all ewes. (b) Linear regression for BWG in relation to STD of DFC% for the Medium Consistency group.

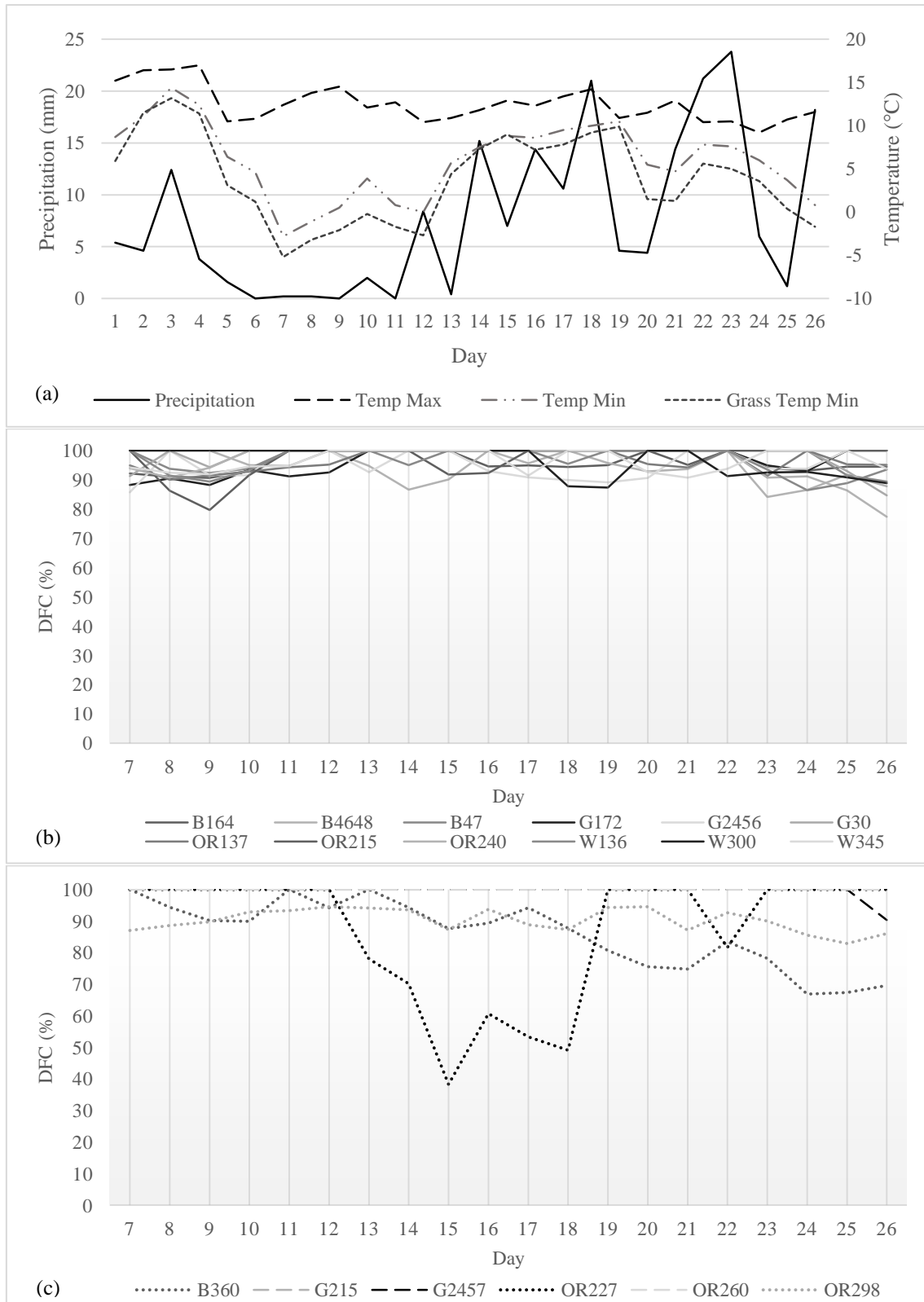


Figure 8. Degrees of Functional Coupling (DFCs) for the activity of ewes over the autumn period. DFC% was calculated for moving seven-day intervals, thus, the graphic starts at the 7th day. (a) Absolute values for weather over the autumn period. (b) DFC% response of 18 ewes that did not present deviation from the population intercept and slope over the autumn period (Medium Consistency group). (c) DFC%

835 response of six ewes that presented deviation from the population intercept over the autumn period. These
836 ewes differed from the population by an individual level that is independent of the temperature. The three
837 ewes represented by dashed lines presented higher consistency of DFC% (High Consistency group),
838 whereas the three ewes represented by dotted lines showed the lower consistency of DFC% (Low
839 Consistency group).